CHANGES IN DENSITY, WEIGHT, CHLORIDE, AND SWIMBLADDER GAS IN THE KILLIFISH, FUNDULUS HETEROCLITUS, IN FRESH WATER AND SEA WATER

VIRGINIA S. BLACK

Department of Biology, Dalhousie University, Halifax

INTRODUCTION

The field of osmotic regulation in aquatic animals has received much attention during the last fifty years. In fish this work has been largely directed toward a study of the euryhaline species such as the eel and salmon (Krogh, 1939). The theories currently accepted for the maintenance of water and salt balance by normal fish in sea water and fresh water were first thoroughly reviewed by Smith (1932), and have been well summarized by Krogh (1939), Baldwin (1940), and Scheer (1948).

The killifish, *Fundulus,* has been used by many investigators, probably because it is one of the few small euryhaline genera which are available in quantity and adapt readily to aquarium life. The most extensive work dealing with the effect of density changes on *Fundulus* and other fish was carried out by Sumner (1905). His experiments are based mainly on viability of groups of fish in various salinities and fresh water. He also measured weight changes and changes in chloride in the water and in the tissues of *Fundulus* resulting from removal from sea water to fresh water and *vice versa.*

The present investigation was designed to obtain serial quantitative measurements of rapid adjustments of a marine fish to fresh water, and so construct a more complete picture of a reaction whose qualitative aspects are already known.

The author is deeply indebted to Dr. F. R. Hayes for invaluable assistance in formulating the problem and also for information and helpful suggestions regarding the measurements of density of fishes.

MATERIAL AND GENERAL PROCEDURE

Live specimens of *Fundulus heteroclitus,* commonly known as killifish, mummichog, or salt water "minnow," were obtained from salt water flats northeast of Halifax, Nova Scotia. This species is normally found in the sea, in estuaries, and in brackish waters. In the laboratory the fish were kept in large glass aquaria (10 inches by 17.5 inches) having a depth of water of 6.5 inches. Stock sea water was obtained from the Northwest Arm, Halifax. The tap water was derived from the Halifax civic water supply. An analysis of the water made in 1940 (Leverin, 1942) from samples at the pumping station is given in Table I.

Experiments were not begun until the fish had been in the laboratory for at least two days. Fish which had been in the laboratory more than a week were not used to begin a series of experiments. The fish were not fed during the period of the experiment, but stock fish were fed every two days on Aylmer's canned beef

TABLE I

Analysis of Halifax water supply, July 1940 (Leverin, 1942)

	Parts per million		Parts per million
Color	30.0	Bicarbonate (HCO_3)	None
Alkalinity as $CaCO_3$	None	Sulphate (SO_4)	6.6
Residue on evaporation dried at 110° C.	30.0	Chloride (Cl)	2.5
Silica (SiO_2)	4.0	Nitrate (NO_3)	0.35
Iron (Fe)	0.05	Total hardness as $CaCO_3$	18.8
Calcium (Ca)	5.7	Calcium hardness	14.3
Magnesium (Mg)	1.1	Magnesium hardness	4.5

TABLE II

Density, weight, chloride and swimbladder gases of F. heteroclitus *in sea water*

Date 1947	Sex	Weight grams	Density (see text)	Chloride m.eq./kilo wet tissue	Swimbladder		
					CO_2 %	O_2 %	Vol. swimbladder / Wt. of fish
Series I							
June 2	♂	8.28		42	6.2	11.9	0.060
	♀	6.34			6.0	10.1	0.040
	♀	4.50		58	2.3	13.1	0.060
4	♀	6.98		55	3.1	10.5	0.063
	♂	7.87		58	1.3	14.2	0.051
7	♀	6.86		66	0	12.6	0.044
	♀	7.15		60	2.7	10.2	0.050
9	♀	3.40	1.017	50	1.2	15.5	0.048
10	♀	6.90	1.027	55	0.6	12.1	0.061
Average		*6.5*		*56*	*2.6*	*12.2*	*0.053*
Series II							
July 21	♀	2.09		50	1.2	15.1	0.033
	♀	1.51		50			
	♂	4.43			3.6	17.3	0.056
	♀	10.18			3.2	14.0	0.054
	♂	1.87			2.3	21.4	0.029
22	♀	1.47		50	3.0	20.0	0.029
23	♀	1.67		55	1.1	15.1	0.031
	♀	1.26	1.026	52	0.8	12.5	0.032
	♂, ♀	1.74		46			
24	♂	1.71		51	1.3	17.5	0.039
Aug. 1	♀	1.54		58	0.5	11.8	0.045
	♀	4.72	1.024	60	0.8	10.7	0.044
	♂	1.00		60	0.2	11.9	0.045
Average		*2.7*		*53*	*1.6*	*15.2*	*0.040*

prepared for babies. Both sexes were used. Most of the fish were sexually mature. The range in weight of the fish was one to twelve grams, but an attempt was made to select fish of approximately the same weight for each series.

In all aquaria the water was circulated and aerated by fine streams of air bubbles. The water was maintained at a pH between 7.0 and 8.5. The temperature varied from 15° C. to 19° C., but changes were taken into account in the density determinations.

Weight and density measurements were made on groups of two or three fish, taken as a unit, before the fish were transferred from sea water to the tap water (soft water) aquarium. The transition from sea water to fresh water, or the reverse, was always direct; no gradual acclimatization was undertaken. After a known number of hours or days the weight and density measurements were again made. The groups of fish were identified by the manner in which the tail fin was clipped.

Swimbladder gases and chloride content of the fish in the sea water aquarium were determined every other day during the course of a series of experiments and provided the data for "normal sea water fish" (Table II). Swimbladder gases and chloride content of the experimental fish in tap water were determined immediately after the weight and density measurements had been made.

Methods

All weight determinations were made on a chemical balance accurate to 0.01 gram. The fish were blotted with paper towels in order to remove excess water before weighing.

Dry weights are given in Table III. The fish were dried in an electric oven for 14 to 20 hours at 95° C. and then for 1 to 2 hours at 105° to 115° C.

TABLE III

Dry weights of Fundulus heteroclitus

Date 1947	Chloride in water m.eq./liter	Sex	Weight grams	Dry weight % of wet weight
Aug. 6	440 (sea water)	♀	2.20	22.0
		♀	2.28	20.8
		♀	1.06	20.8
		♂	1.05	20.9
		♂	2.52	21.0
				Average 21.1
	After 7 hrs. in less than 1 (tap water)	♀	1.48	19.4
		♂	1.40	20.0
				Average 19.7
		% water taken on in 7 hrs. = 6.6		

The method of Lowndes (1938) was employed to determine the density of the fish. The same weighing bottle (51.27 cc.) was used in all determinations. The method is based on the fact that density of fish $= \frac{\text{weight of fish}}{\text{volume of fish}}$. The weight of the fish was obtained both directly and by calculation from weight in water and volume in water. The volume of the fish in sea water was calculated from a comparison of the chloride content of undiluted sea water with the chloride content of the water which had been decanted from the weighing bottle containing the fish and made up to volume with distilled water.

The volume of fish in fresh water was determined colorimetrically at the suggestion of Dr. Hayes. Measurements were made using aquarium water dyed with trypan blue. The volume was determined by colorimetric comparison of water in the volumetric flask (decanted from weighing bottle with fish and made up to volume) and undiluted dyed aquarium water. A Klett colorimeter was used.

Results obtained by the chloride and colorimetric methods were checked by calculating the density from weight in air and weight in water where density of fish $= \frac{\text{weight in air} \times \text{density of water}}{\text{weight in air} - \text{weight in water}}$. These three procedures for determining and calculating densities were carried out on six separate groups of fish in dyed sea water. The standard error for results from the three methods varied from ± 0.0002 to ± 0.0055 in the six analyses made, showing good agreement of the methods.

After weight and density had been determined the swimbladder gas was withdrawn under water using a hypodermic needle and syringe. The gas was immediately expelled into a Krogh micro-gas-analyzer (Krogh, 1908). The volume of gas was estimated in the bulb of the apparatus which was calibrated in tenths of a cubic centimeter. Estimates of small gas volumes were less reliable than those of large volumes. About 0.08 cc. of the gas was analyzed, carbon dioxide being absorbed by one-fourth normal potassium hydroxide, and oxygen by reduced violet chrome alum, as described by Hayes (1939).

Chloride in water samples was determined by Mohr's method. The chloride content of fish tissues was analyzed by means of Van Slyke and Sendroy's micromethod as given by Peters and Van Slyke (1932). All fish were washed under the tap before chloride analyses were made.

Results and Discussion

The results reported here are derived from work done in June and July 1947. Two preliminary series (August 1946; May 1947) were also made for which the data are not complete but which show clearly the same trends indicated in the two series presented in this paper. A fifth series in which fish were transferred to tap water containing calcium carbonate gave similar results, although the loss of salt and gain in weight (water) was not as marked (cf. Weil and Pantin, 1931; Pantin, 1931; Breder, 1933).

Series I. *Response of Fundulus heteroclitus to soft tap water*

The average density of the sea water was 1.019, and of the fish in sea water, 1.023 (± 0.0014 for 16 determinations). This average figure for density of fish in sea water is derived from density determinations on fish before introduction to tap water of density 0.997. The course of adjustment is presented in Figure 1. Each point in Figures 1A, 1B, and 2 represents an average of two or three determinations, except points at 18 hours, 72 hours, and 7 days where only one analysis was made. In Figure 1C each point indicates a single density determination using one, two, or three fish.

Within 12 hours the density of the fish decreased to 1.001 and remained between 0.995 and 1.006 for the duration of the experiment. The slight rise in density at 24 hours occurred in both series (Figs. 1C, 3) and might reflect the rapid loss of weight (water) at this time (Figs. 1B, 3).

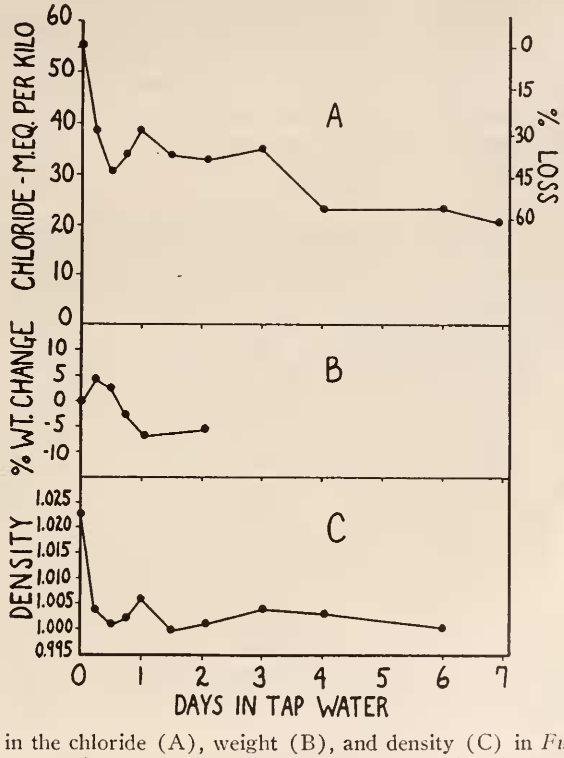

FIGURE 1. Changes in the chloride (A), weight (B), and density (C) in *Fundulus heteroclitus* when transferred directly from sea water to soft tap water.

A rapid gain in weight (4 per cent in 6 hours) follows the introduction of *Fundulus* to fresh water. This phenomenon has been known for some time and Sumner (1905) and Scott (1910) observed an initial gain followed by a loss which persisted throughout the experiment as a result of inanition. The rapid intake of water, probably by way of the gills, is a natural osmotic response to the hypotonic environment. Measurements of the dry weight of two fish at the height of weight gain (7 hours) show a 6.6 per cent increase in water content (Table III). Excretion of water by the kidney may account for the decrease in weight which begins after 8 hours, returning to the initial weight level after about 15 hours and continuing to decrease at a rapid rate for the rest of the first day.

The change in chloride content of the fish is shown in Figure 1A. It should be emphasized that these chloride results apply to the whole fish, and do not reflect the response of any single tissue. By referring to Figures 1A and 1B, it will be seen that an initial dip in the chloride curve corresponds to the curve showing a gain and loss in weight. Since the chloride measurements are calculated on the basis of weight it is likely that the irregularity in the chloride curve during the first day is not real but that chloride is being lost at a constant rate during the rapid gain and loss of water. Sumner (1905) was also aware of this possibility. He found a 25 per cent loss of chloride in *F. heteroclitus* after one day in water having a density of 1.000 or 1.001. The present investigation shows a loss of 30 per cent chloride in one day in water of density 0.997. After the first day chloride continues to be lost gradually during the following 6 days. The fish began to die after the second day, although mortality was not high during the course of the experiments. Of 21 fish in the series, 3 died within the 7 days of experiment. Lack of food may have been a significant factor, although none of the fasting control fish died. Plankton in the unfiltered sea water was available to the controls and no extra demand was being made on their energy as was the case for fish adjusting to fresh water.

From Figure 1 it may be concluded that transfer of *Fundulus* to tap water causes a marked decrease in the density of the fish, an initial increase in weight followed by a decrease, and a gradual loss of tissue chloride. As would be expected in a model osmosis experiment, the water passes through the semipermeable membrane (gills) from the less dense (fresh water) to the more dense (body fluids) solution. Salts (chloride) in the body fluids appear to diffuse out into the water. Sumner (1905) was able to detect this increase in the chloride in fresh water after fish had been introduced from sea water. With regard to water and salts there seems to be little physiological regulation for the first 8 hours by fish introduced directly from sea water to fresh water (Fig. 1). After this period the loss of weight throughout the rest of the first day indicates that the kidneys are probably excreting the excess water taken on by the tissues. Gradual loss of chloride continues throughout the 7 days of the experiment. These changes in salt and water content of the fish contribute to a decrease in the density of the animal. Scott (1910) reported a decrease in the density of the blood of *F. heteroclitus* from 1.0510 to 1.047 after about 8 hours in fresh water. The density of the whole fish at this time, however, has decreased from 1.023 to approximately 1.003 (Fig. 1C).

Adjustment of the density of the fish to fresh water is also assisted by the deposition of gases into the swimbladder, thus making the animal lighter. The function of the swimbladder as an organ for the maintenance of buoyancy in fish is well known, although the exact mechanism whereby fish can separate gases from the blood and deposit them in the swimbladder is still imperfectly understood (Rauther, 1937). The importance of the swimbladder in determining the density of fish is clearly indicated in a paper by Andriaschev (1944) who took density measurements of eight genera of Black Sea fish possessing swimbladders, and eight genera without swimbladders. The range of densities for fish with swimbladders was 1.012–1.021; those without ranged from 1.061 to 1.085.

When *Fundulus* is transferred from sea water directly to tap water, each fish sinks immediately to the bottom for it is heavier than the water. Gas secretion begins and buoyancy is regained after about 24 hours. The equipment for gas secretion and resorption in *Fundulus* consists of a capillary network and "gas gland." The swimbladder of *Fundulus* is physoclistous, i.e., without an open duct leading to the esophagus. Hence all changes in gas content presumably take place by way of the blood.

An inspection of Figure 2C will show that the volume of gas, measured at barometric pressure, increases about 50 per cent in 24 hours. This increase in gas has the effect of inflating the fish and thus decreasing its density. The greatest part of the secreted gas appears to be oxygen which becomes 175 per cent of normal in 24 hours (Fig. 2A). Carbon dioxide increases somewhat in the first 12 hours,

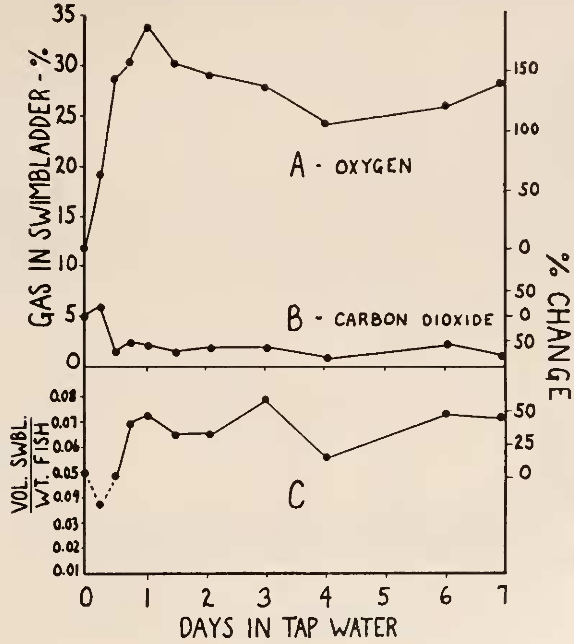

FIGURE 2. Changes in the swimbladder gas of *Fundulus heteroclitus* when transferred directly from sea water to soft tap water.

decreases again before 24 hours, but shows no marked change thereafter (Figs. 2B, 4).

The data pictured in Figure 2 (and Fig. 4) show that *Fundulus* from sea water adjust to the less dense tap water by increasing the volume of gas in the swimbladder. The greatest part of the secreted gas is oxygen.

Series II. *Response of Fundulus heteroclitus to sea water after two days in soft tap water*

A second series of experiments was run to determine the response to sea water of a group of fish whose fresh water history was known. When the fish are returned to sea water they behave like tops and spin around, head down, at an angle of 45° for about half an hour. The spinning effect is due to the rapid fin movement in an attempt to swim down since they are much lighter than the sea water as a result of the increase in gas volume in fresh water.

The response of the fish on return to sea water is immediate and rapid. The complete history of these fish with respect to change from sea water to fresh water, and the reverse, is presented in Figures 3 and 4. The weight and density measurements on the graphs represent single determinations using two or three individuals. Each point for chloride content and swimbladder gases is the average of results from two fish in the tap water series; in the sea water series, each point represents one fish. The broken line in Figure 3 indicates that the shape of the curve after 24 hours is taken from Figure 1B. In Figure 4 the initial part of the oxygen

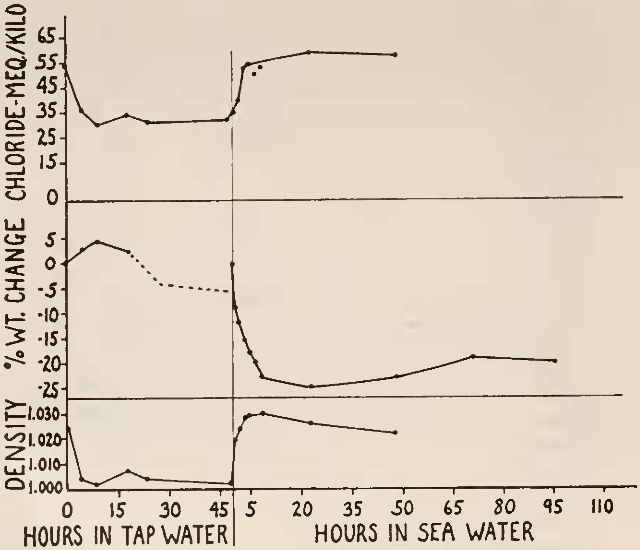

FIGURE 3. Changes in chloride, weight, and density in *Fundulus heteroclitus* transferred from sea water to soft tap water for 2 days, then returned to sea water.

graph is shown by a broken line because of the large difference between the data whose average is presented by the second point (29 per cent and 44 per cent).

The density, chloride, and swimbladder gases return to normal in 4 hours, although it took 12 to 24 hours for the change from sea to fresh water. Loss of weight, due largely to loss of water, appears to be a continuous process reaching a maximum of − 25 per cent. The rate of passage of water out of the fish is three to four times greater than the rate of imbibition when the fish is put into tap water from sea water. The entire adjustment of the fish to the sea water takes place in six hours, or about four times as quickly as the reverse adjustment from sea water to fresh water.

This difference in rate of change of the factors measured might be explained as follows. When the fish is transferred to fresh water the kidneys may start to function immediately, although the effect of their work is not evident until after

8 hours. If water is being excreted by the kidneys during the period of weight gain the net gain would be the difference between water taken on by the tissues and water excreted by the kidneys. When the fish is returned to sea water, however, the water is passing out of the fish both at the gills and kidneys so that the weight (water) loss could be accomplished more rapidly. Some water, however, probably enters with the chloride.

It is difficult to explain the difference in rate of movement of chloride unless the slower loss of chloride in fresh water is due to the functioning of a salt conservation mechanism in the gills. Krogh has shown that several species of fresh water fish can extract salts from fresh water (Krogh, 1939). A similar mechanism may be functioning here so that the fish can in some way regain a little of the chlo-

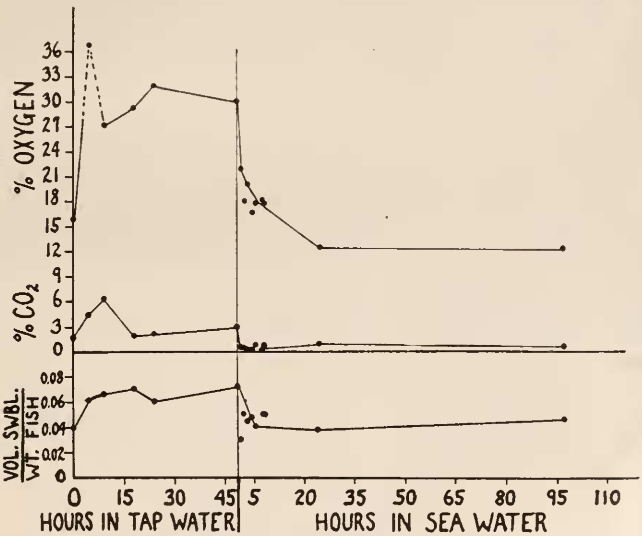

FIGURE 4. Changes in swimbladder gas of *Fundulus heteroclitus* transferred from sea water to soft tap water for 2 days, then returned to sea water.

ride, and so decrease the net chloride loss. When the fish returns to sea water, however, the gradient is such that chloride passes into the fish until the normal salt content is regained.

On the other hand, the gills may not be equally permeable in both directions; or the fish may have some means of controlling movement of water and salts by neural and hormonal activity in a manner similar to that described for the adrenal cortex of dogs (Loeb *et al.*, 1933) and pituitary of frogs (Boyd and Whyte, 1938).

The difference between the time required for the separation of gases from the blood, to increase the gas volume in the swimbladder in fresh water, and that necessary to resorb the gases on returning to sea water might be explained by assuming that the active secretion of gases into the swimbladder would demand more time and energy than the passive resorption of the gases by the blood.

When experiments of the type described in this paper are applied to fish in various stages of acclimatization, a graphic description of the process would then be available which might facilitate further work on the mechanisms involved in acclimatization to salinity changes.

Summary

1. The adjustment of the killifish, *Fundulus heteroclitus*, to fresh water involves significant changes in the swimbladder gas, chloride content, weight, and density of the fish. Adaptation to fresh water is complete after 24 hours.

2. The gain in weight is only temporary, returning to normal after 18 hours The gain is due to taking on water from the hypotonic medium. The subsequent loss may be the result of kidney excretion and inanition.

3. The chloride decreases markedly during the first 12 hours but some appears to be regained after 24 hours. This irregularity corresponds to the short period of weight gain and loss, and appears because the water taken on by the fish is included in the calculation of chloride on the basis of weight. After 4 days in fresh water the fish have lost approximately 60 per cent of their normal chloride content.

4. When the fish are first put into fresh water they sink immediately to the bottom because fresh water is less buoyant than sea water. In order to adjust to the change and regain normal buoyancy the fish deposit oxygen and, to a lesser degree, carbon dioxide into the swimbladder. The volume of gas measured at barometric pressure is greater after adjustment to fresh water, showing that the amount of gas in the swimbladder has increased.

5. All the above adjustments tend to decrease the density of the fish to approximately the density of the water within 24 hours.

6. When fish are replaced in sea water after two days in fresh water, they regain their normal chloride, density, and swimbladder gas within six hours, or four times as fast as the previous adjustment to fresh water. Possible reasons for this difference in rate of adjustment are discussed.

LITERATURE CITED

Andriaschev, A. P., 1944. Determination of natural specific gravity of fish. *Compt.-rend. Acad. Sci. URSS*, **43**: 80–82.

Baldwin, E., 1940. *An introduction to comparative biochemistry.* Cambridge. 120 pp.

Boyd, E. M., and D. W. Whyte, 1938. The effect of extract of the posterior hypophysis on the loss of water by frogs in a dry environment. *Amer. Jour. Physiol.*, **124**: 759–766.

Breder, C. M., 1933. The significance of calcium to marine fishes invading fresh water. *Anat. Rec.*, **57** (Supplement): 57.

Hayes, F. R., 1939. A pipette for the micro estimation of respiratory gases. *Proc. N.S. Inst. Sci.*, **19**: 373–388.

Krogh, A., 1908. On micro-analysis of gases. *Skand. Arch. f. Physiol.*, **20**: 279–288.

Krogh, A., 1939. *Osmotic regulation in aquatic animals.* Cambridge. 242 pp.

Leverin, H. S., 1942. Industrial waters of Canada. *Report on Investigations 1934 to 1940.* Ottawa. 112 pp.

Loeb, R. F., D. W. Atchley, E. M. Benedict and J. Leland, 1933. Electrolyte balance studies in adrenalectomized dogs with particular reference to the excretion of sodium. *Jour. Exp. Med.*, **57**: 775–792.

Lowndes, A. G., 1938. The density of some living aquatic organisms. *Proc. Linnaean Soc. London*, 150th session, Part 2: 62–73.

PANTIN, C. F. A., 1931. The adaptation of *Gunda ulvae* to salinity. III. The electrolyte exchange. *Jour. Exp. Biol.*, **8**: 82–94.

PETERS, J. P., AND D. D. VAN SLYKE, 1932. *Quantitative clinical chemistry.* Volume II, Methods. Baltimore, Williams & Wilkins Co.

RAUTHER, M., 1937. Die Schwimmblase. *Handbuch der vergleichenden Anatomie der Wirbeltiere,* **3**: 883–908.

SCHEER, B. T., 1948. *Comparative physiology.* New York, Wiley & Sons. 563 pp.

SCOTT, G. G., 1910. Effects of changes in the density of water upon the blood of fishes. *Bull. U. S. Bur. Fish.*, **28**: 1143–1150.

SMITH, H. W., 1932. Water regulation and its evolution in the fishes. *Quart. Rev. Biol.*, **7**: 1–26.

SUMNER, F. B., 1905. The physiological effects upon fishes of changes in the density and salinity of the water. *Bull. U. S. Bur. Fish.*, **25**: 53–108.

WEIL, E., AND C. F. A. PANTIN, 1931. The adaptation of *Gunda ulvae* to salinity. II. The water exchange. *Jour. Exp. Biol.*, **8**: 73–81.